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IL-4 polymorphism influences susceptibility to *Pneumocystis jirovecii* pneumonia in HIV-positive patients

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Abstract: **OBJECTIVES** *Pneumocystis jirovecii* pneumonia (PJP) is an important cause of morbidity and mortality in HIV-positive patients. Polymorphisms in immune genes are increasingly reported to influence susceptibility to fungal infections. We analysed the role of 21 single nucleotide polymorphisms from 19 candidate genes on PJP development in patients from the Swiss HIV Cohort Study. **DESIGN AND METHODS** The analysis included patients with a nadir CD4 T-cell count less than 200 cells/l, divided into a discovery (N = 1645) and a replication (N = 1861) cohort. The associations were analysed by using cumulative incidence curves as well as competing risk regression over 18 years, starting from the estimated date of HIV infection, considering death a competing risk, with censoring at lost follow-up, and assuming the dominant mode of inheritance. **RESULTS** The minor allele of rs2243250 in IL-4 was associated with the risk of PJP in the discovery cohort (cumulative incidence 0.18 versus 0.12, P = 0.002). This association was replicated in the validation cohort (0.16 versus 0.12, P = 0.02). It was still significant in multivariate models, adjusted for HIV transmission mode, viral load, CD4 T cells slope, age, antiretroviral therapy, tobacco smoking, hepatitis C virus coinfection, year of cohort entry and PJP prophylaxis (global subhazard ratio 1.42, 95% confidence interval 1.17-1.73, P = 0.0004). **CONCLUSION** Our data suggest rs2243250, a single nucleotide polymorphism known to influence IL-4 production, is associated with susceptibility to PJP in HIV-positive patients.

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IL-4 polymorphism influences susceptibility to *Pneumocystis jirovecii* pneumonia in HIV-positive patients

Agnieszka Wójtowicz^a, Stéphanie Bibert^a, Patrick Taffé^b,
Enos Bernasconi^c, Hansjakob Furrer^d, Huldrych F. Günthard^{e,f},
Matthias Hoffmann^g, Michael Osthoff^h, Matthias Cavassini^{a,*},
Pierre-Yves Bochud^{a,*}, the Swiss HIV Cohort Study[†]

Objectives: *Pneumocystis jirovecii* pneumonia (PJP) is an important cause of morbidity and mortality in HIV-positive patients. Polymorphisms in immune genes are increasingly reported to influence susceptibility to fungal infections. We analysed the role of 21 single nucleotide polymorphisms from 19 candidate genes on PJP development in patients from the Swiss HIV Cohort Study.

Design and methods: The analysis included patients with a nadir CD4⁺ T-cell count less than 200 cells/ μ l, divided into a discovery ($N = 1645$) and a replication ($N = 1861$) cohort. The associations were analysed by using cumulative incidence curves as well as competing risk regression over 18 years, starting from the estimated date of HIV infection, considering death a competing risk, with censoring at lost follow-up, and assuming the dominant mode of inheritance.

Results: The minor allele of rs2243250 in IL-4 was associated with the risk of PJP in the discovery cohort (cumulative incidence 0.18 versus 0.12, $P = 0.002$). This association was replicated in the validation cohort (0.16 versus 0.12, $P = 0.02$). It was still significant in multivariate models, adjusted for HIV transmission mode, viral load, CD4⁺ T cells slope, age, antiretroviral therapy, tobacco smoking, hepatitis C virus coinfection, year of cohort entry and PJP prophylaxis (global subhazard ratio 1.42, 95% confidence interval 1.17–1.73, $P = 0.0004$).

Conclusion: Our data suggest rs2243250, a single nucleotide polymorphism known to influence IL-4 production, is associated with susceptibility to PJP in HIV-positive patients.

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^aDepartment of Medicine, Infectious Diseases Service, ^bInstitute for Social and Preventive Medicine, University Hospital and University of Lausanne, Lausanne, ^cDivision of Infectious Diseases, Regional Hospital of Lugano, Lugano, ^dDepartment of Infectious Diseases, Bern University Hospital and University of Bern, Bern, ^eDivision of Infectious Diseases and Hospital Epidemiology, University Hospital Zürich and University of Zürich, ^fInstitute of Medical Virology, University of Zürich, Zürich, ^gDivision of Infectious Diseases and Hospital Epidemiology, Department of Internal Medicine, Cantonal Hospital St.Gallen, St.Gallen, and ^hDivision of Infectious Diseases and Hospital Epidemiology and Department of Internal Medicine, University Hospital Basel, Basel, Switzerland.

Correspondence to Pierre-Yves Bochud, Infectious Diseases Service, Department of Medicine, University Hospital and University of Lausanne (CHUV), Rue du Bugnon 46, CH-1011 Lausanne, Switzerland.

Tel: +41 21 314 43 79; fax: +41 21 314 10 33; e-mail: Pierre-Yves.Bochud@chuv.ch

* Matthias Cavassini and Pierre-Yves Bochud contributed equally to the article.

[†] Members of the Swiss HIV Cohort Study are indicated in Acknowledgments section.

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Introduction

Pneumocystis jirovecii is an opportunistic fungus causing severe pneumonia in HIV/AIDS and other populations of immunosuppressed patients [1]. It is one of the most common AIDS-defining conditions and an important cause of AIDS-related deaths [2–4]. *P. jirovecii* pneumonia (PJP) typically manifests among individuals with a CD4⁺ T-cell count of less than 200/μl, in particular when the HIV viral load is elevated [5]. Other factors such as ethnicity [6] and HIV transmission mode [7] have been reported to alter susceptibility to PJP in some studies, but this was not universally confirmed [8].

Increasing evidence suggests that polymorphisms in host immune genes influence the course of infections due to fungal pathogens. Single nucleotide polymorphisms (SNPs) in genes encoding pattern recognition receptors (PRRs) such as pentraxin 3 [9–11] and Dectin-1 [12–14] are emerging as reliable predictors of the future occurrence of invasive aspergillosis among onco-hematological patients as well as hematopoietic stem cell and solid organ transplant recipients [15,16]. Similarly, polymorphisms in genes encoding cytokines were associated with both invasive aspergillosis (IL1B [17,18]) and candidiasis (TNFα [19], IL-4 [20]). Fewer studies examined the role of immune gene polymorphisms in susceptibility to PJP or AIDS progression. One study associated low producing mannose binding lectin 2 (MBL2) haplotype [21] with PJP infection in a small cohort of HIV-infected individuals. Other polymorphisms apparently associated with PJP in HIV-positive patients are in fact markers of rapid progression to AIDS [22–24].

In this study, we analysed the role of polymorphisms from 21 candidate genes encoding relevant fungal PRRs and cytokines/chemokines with regards to the predisposition to PJP in the patients from the Swiss HIV Cohort Study (SHCS).

Methods

Study cohort and design

The SHCS (www.shcs.ch) is a prospective observational multicenter cohort of seven Swiss hospitals (Basel, Bern, Geneva, Lausanne, Zurich, Lugano and St. Gallen [25]). More than 20 000 HIV-infected patients have been enrolled in Switzerland since 1988 [25,26]. The clinical stage of the patients was defined according to the 1993 classification system for HIV infection of the Centers for Disease Control and Prevention [27]. Demographic characteristics including age, duration of HIV infection, CD4⁺ T-cell count nadir, opportunistic infections, HIV maximal viral load and antiretroviral therapy used were extracted from the SHCS clinical database [28]. Written

informed consent was obtained from all patients, including consent for the genetic studies. All patients whose CD4⁺ T-cell count was of less than 200 cells/μl for at least 3 months were selected. Patients were randomly stratified into a discovery group and a validation group at a 1 : 1 ratio. Additional patients who were entered into the cohort after the randomization process were added to the validation group.

Definite and presumptive PJP infections were defined according to standard definitions [29]. Briefly, a definitive diagnosis required the identification of the pathogen from respiratory samples by cytology/microscopy or histology. The presumptive diagnosis was made on a combination of clinical signs/symptoms and radiological findings (<http://www.shcs.ch/122-4-cdc-category-c-diagnoses#4.2.1>). The CD4⁺ T-cell loss rate was calculated for each individual using a linear regression of time on the square root of CD4⁺ T-cell counts as described elsewhere [30]. Unknown HIV-infection dates were estimated by using a joint back calculation model as described elsewhere [31].

Genotyping

A total of 21 SNPs from 19 genes were selected based on a systematic literature review, including SNPs previously associated with fungal infections. Genomic DNA was extracted from cell pellets or whole blood with use of a MagNA Pure LC DNA Isolation Kit (Roche Applied Science, Munich, Germany) according to the manufacturer's protocols. The SNPs were part of a customized Golden Gate Genotyping Assay (Veracode technology, Illumina) or were genotyped using a Competitive Allele Specific PCR system (KBioscience/LGC Genomics; <http://www.lgcgenomics.com>). Genotype data were analyzed on a BeadXpress Reader or a KusterKaller software (KBioscience/LGC Genomics) according to the standard protocols and quality controls [32].

Statistical analysis

Statistical analyses were performed in Stata 15.1 (StataCorp LLC, College Station, Texas, USA). Cumulative incidence of PJP was assessed over a 18 years period starting at the estimated date of the HIV infection with censoring at last follow-up and considering death as a competing event, by using *stcrreg* implemented in Stata. For simplicity a dominant mode of inheritance was assumed for each SNP and the first episode of PJP was considered. Multivariate analyses were performed by using *stcrreg*, with adjustment for co-variables possibly associated with PJP, considering a cut-off *P* value of 0.1 in the univariate analyses. CD4⁺ T-cell counts were accounted for either by using the CD4⁺ slope before antiretroviral therapy (as described above) or as a time-varying covariable. Other variables such as hepatitis C virus (HCV) or hepatitis B virus infection, as well as antiretroviral and anti-*Pneumocystis carinii* pneumonia (PCP) drugs were accounted for either as present/absent at any time during follow-up (e.g demographic tables) or

as time-varying covariables (time-dependent analyses). Associations were first analysed among patients from the discovery cohort and, when significant, replicated in the validation cohort. The linkage disequilibrium and Hardy–Weinberg equilibrium (HWE) tests were assessed by using the *plink* and *hwe* softwares implemented in Stata. Bonferroni's correction was used to adjust data for the number of tests included in the models. MBL2 haplotypes were phased using PHASE software version 2.1 (University of Washington, Seattle, Washington, USA).

Results

A total of 3506 Caucasian individuals were included (1645 in the discovery and 1861 in the replication study, Table 1), among whom 470 developed PJP (413 definite and 57 presumptive). Patient characteristics were equally distributed in the discovery and the replication studies, with a mean age of 33 years (range 10–74) at time of cohort entry, a male predominance (77%), a mean CD4⁺ T-cell nadir count of 90.5 cells/ μ l (range 0–199) and a mean maximal log viral load of 5.20 copies/ml (range 1–8). HIV infection was acquired by male–male sexual contact in 40%, by heterosexual contact in 31% and by intravenous drug use in 26%.

All the SNPs were at the HWE equilibrium and had minor allele frequencies (MAF) comparable to the ones known for the white population (Supplementary Table S1, <http://links.lww.com/QAD/B490>). In the discovery cohort, associations ($P < 0.05$) were observed for four polymorphisms in four genes, including rs2243250 in IL-4 [cumulative incidence (CI) 0.18 versus 0.12, $P = 0.002$, Fig. 1a], rs4252125 in plasminogen (CI 0.11 versus 0.16, $P = 0.005$), rs16910526 in Dectin-1 (CLEC7A; CI 0.08 versus 0.14, $P = 0.01$) and rs17886395 in surfactant protein A (CI 0.10 versus 0.15, $P = 0.03$, Table 2).

Among those, only one association was significant after Bonferroni correction for multiple testing (21 tests, rs2243250 in IL-4). This association was also significant in the replication cohort (CI 0.16 versus 0.12, $P = 0.02$; Fig. 1b). Furthermore, the association was still significant in a multivariable regression model in both the discovery (subhazard ratio, SHR = 1.43, 95% confidence interval 1.07–1.92, $P = 0.02$) and replication (SHR = 1.42, 95% confidence interval 1.08–1.85, $P = 0.01$, Table 3) studies. In the combined cohorts after adjustment for the maximal HIV viral load, antiretroviral therapy, CD4⁺ slope, age at estimated time of HIV infection, PJP prophylaxis, tobacco use, HCV coinfection, period of cohort entry as well as the mode of HIV transmission, the association was more significant (SHR = 1.42, 95% confidence interval 1.17–1.73, $P = 0.0004$). The association between PJP and rs2243250 were significant when the

Table 1. Demographic characteristic of the patients.

Variable	Discovery, <i>N</i> = 1645	Replication, <i>N</i> = 1861	All patients, <i>N</i> = 3506
	<i>N</i> (%)	<i>N</i> (%)	<i>N</i> (%)
Age at cohort entry (mean years; range)	32.5 (10–73)	33 (13–74)	32.8 (10–74)
Male sex	1273 (77)	1425 (77)	2698 (77)
ART/HAART therapy at any time	1641 (99)	1856 (99)	3495 (99)
HIV maximal viral load (mean RNA log ₁₀ copies/ml; range) ^a	5.20 (2–8)	5.21 (1–8)	5.20 (1–8)
Nadir CD4 ⁺ T-cell count (mean cells/ μ l; range) ^b	89.9 (0–199)	91.0 (0–199)	90.5 (0–199)
CD4 ⁺ slope before ART/HAART initiation (mean; range) ^c	–2.12 (–7–1)	–2.16 (–6–2)	–2.14 (–7–2)
PJP ^d	240 (15)	260 (14)	500 (14)
At presentation	135	162	297
During follow-up	105	98	203
Type of HIV transmission			
Male–male sexual contact	681 (41)	725 (39)	1406 (40)
Heterosexual contact	462 (28)	611 (33)	1071 (31)
Intravenous drug user	446 (27)	462 (25)	908 (26)
Other/unknown	58 (4)	63 (3)	121 (3)
HCV coinfection ^e	551 (33)	616 (33)	1165 (33)
Active HBV infection ^f	64 (4)	82 (4)	146 (4)
Tobacco smokers ^g	985 (60)	1118 (60)	2103 (60)

ART, antiretroviral therapy; HBV, hepatitis b virus; HCV, hepatitis C virus; PJP, *Pneumocystis jirovecii* pneumonia.

^aMean maximal HIV RNA load, was missing in two and seven patients in the discovery and replication cohort, respectively.

^bLowest level of a CD4⁺ T-cell count.

^cRate of CD4⁺ depletion in the absence of HAART, was missing in 24 and 25 patients in the discovery and replication cohort, respectively.

^dAmong PJP cases, 202 (84%) were definitive and 38 (16%) presumptive in the discovery cohort and 237 (91%) definitive and 23 (9%) presumptive in the replication cohort.

^eReflected by HCV serology.

^fHBV serostatus, defined by the presence of HBsAg in the blood.

^gAt cohort entry: more than 10 packet unit year.

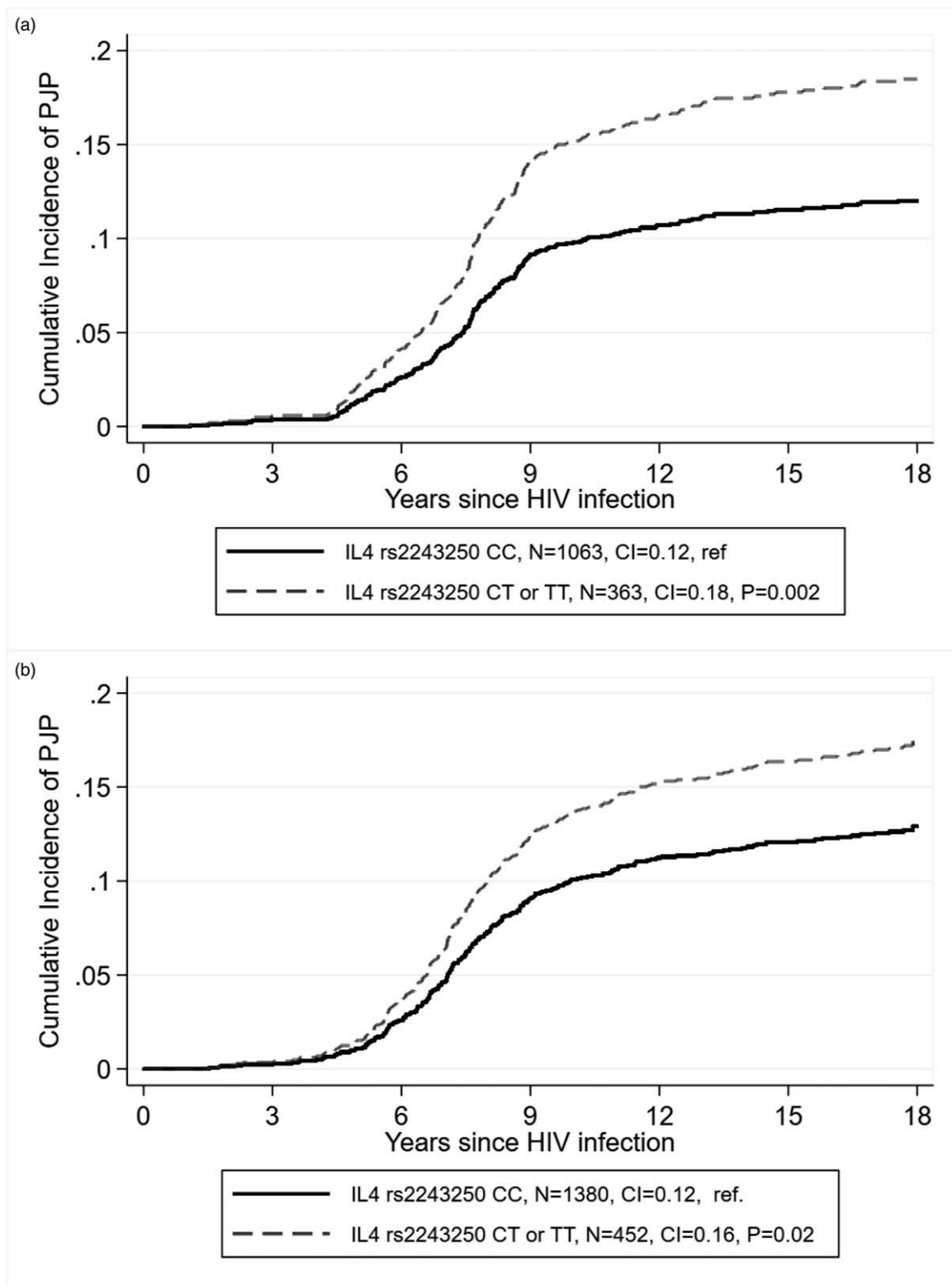


Fig. 1. Cumulative incidence of *Pneumocystis jirovecii* pneumonia according to IL-4 rs2243250 in the discovery [(a) $n = 1426$ patients with available genotypes] and replication [(b) $n = 1832$] studies. Graphs were performed using the cumulative incidence function in *stcurve* after competing risk regression with *stcrred*, considering death as competing risk (Stata).

Table 2. Cumulative incidence of *Pneumocystis jirovecii* pneumonia according to candidate gene polymorphisms in HIV-positive patients from Swiss HIV Cohort Study cohort.

Gene	rs number	nt aa change	MAF	Discovery study, <i>N</i> = 1645				Replication study, <i>N</i> = 1861			
				<i>N</i> ^a	Cum. Incid.		<i>p</i> ^b	<i>N</i> ^a	Cum. Incid.		<i>p</i> ^b
					WT	MUT			WT	MUT	
Pattern recognition receptors											
<i>CLEC7A</i>	rs16910526	Y238X	0.08	1639	0.08	0.14	0.01				
<i>TLR3</i>	rs3775291	L412F	0.29	1632	0.15	0.12	0.1				
<i>TLR1</i>	rs5743611	R80T	0.08	1631	0.15	0.13	0.3				
<i>MBL2</i>	Haplotype	Low MBL	0.27	1548	0.11	0.14	0.3				
<i>PTX3</i>	rs3816527	A48D	0.40	1531	0.13	0.14	0.5				
<i>TLR2</i>	rs5743708	R753Q	0.02	918	0.12	0.14	0.7				
<i>TLR6</i>	rs5743810	S249P	0.36	1623	0.14	0.13	0.8				
<i>TLR1</i>	rs5743604	S602I	0.33	1622	0.13	0.13	0.8				
<i>TLR4</i>	rs4986790	D299G	0.05	1623	0.13	0.13	0.9				
Cytokines/Chemokines and other genes											
<i>IL4</i>	rs224333250 ^c	−590 C/T	0.14	1426	0.18	0.12	0.002 ^d	1832	0.16	0.12	0.02
<i>PLG</i>	rs4252125	D472N	0.31	1632	0.11	0.16	0.005 ^e	1839	0.14	0.12	0.2
<i>SPA2</i>	rs17886395	A91P	0.14	1590	0.10	0.15	0.03				
<i>IL1A</i>	rs1800587	−889 C/T	0.28	1598	0.15	0.12	0.05				
<i>TNFα</i>	rs1800629	−308 G/A	0.13	1465	0.11	0.14	0.08				
<i>IL1B</i>	rs1143627	−31 T/C	0.34	1633	0.12	0.15	0.1				
<i>IL4RA</i>	rs1805015	S503P	0.15	1624	0.15	0.12	0.1				
<i>IL19</i>	rs1800896	−1082 A/G	0.44	1634	0.13	0.14	0.5				
<i>CXCL10</i>	rs3921	1642 G/C	0.43	1634	0.13	0.14	0.6				
<i>DEFB1</i>	rs1800972	−44 C/G	0.19	1612	0.14	0.13	0.7				
<i>FCGR2A</i>	rs1801274	R131H	0.48	1614	0.14	0.13	0.9				

CI, confidence interval; CLEC7A, C-type lectin domain 7, also known as Dectin-1; CXCL10, CXC-chemokine ligand-10; DEFB1, human beta-defensin 1; FCGR2A, Fc Fragment of IgG receptor IIa; HR, hazard ratio; IL, interleukin; IL4RA, IL4 receptor subunit alpha; LD, linkage disequilibrium; MAF, minor allele frequency; MBL2, mannose binding lectin 2; PJP, *Pneumocystis jirovecii* pneumonia; PLG, plasminogen; PTX3, pentraxin 3; SHCS, Swiss HIV cohort study; SPA2, surfactant protein A2; TLR, Toll-like receptor; WT, wild type.

^aN stands for the number of available genotypes for each SNP (after quality testing).

^bAssociations were analysed by using *stcrreg*, considering dominant mode of inheritance (patients homo- and heterozygous for the rare allele are compared to the others).

^cBecause some genotypes were missing, the association was also run for rs2070874, which is in strong LD with rs2243250 ($R^2 = 0.96$). The *P* value for rs2243250 was 0.0008.

^d $P = 0.047$ and $P = 0.016$ for rs2243250 and rs2070874, respectively, after Bonferroni correction (21 tests).

^e $P = 0.099$ after Bonferroni correction (21 tests).

presumptive PJP cases were removed from the model (SHR = 1.36, 95% confidence interval 1.10–1.68, $P = 0.004$), and when CD4⁺ T cells were accounted for as a time-dependent covariates instead of a slope (SHR = 1.41, 95% confidence interval 1.14–1.75, $P = 0.00016$, Supplementary Table 3, <http://links.lww.com/QAD/B490>).

The association with rs4252125 in plasminogen tended to be associated after corrections for multiple tests (21 tests, $P = 0.1$) but was not replicated.

Discussion

In this study, we show for the first time an association between a SNP in the *IL-4* gene and susceptibility to PJP. This association discovered in a study of 1645 patients was validated in a replication cohort of 1861 individuals. It was still present in multivariate

analyses accounting for potential confounding factors such as CD4⁺ T-cell decline over time. It is further supported by several lines of evidence for a key role of IL-4, a cytokine, in the adaptive immune responses against *P. jirovecii*.

The *IL-4* gene located on chromosome 5q31.1 encodes IL-4, a polyfunctional cytokine produced by activated T cells, type 2 innate lymphoid cells and mast cells, which is involved in adaptive immunity [33]. Its biological activity is mediated through a heterodimeric structured receptor (IL-4R) consisting of IL-4Rα together with either a γ chain (type1 receptor) or a IL13R-α-1 (type2 receptor) molecule (reviewed in [34,35]). IL-4 promotes the differentiation of CD4⁺ T cells into the Th2 phenotype (also mediated by IL-13 and IL-10), leading to B-cell activation and production of neutralizing antibodies such as IgE and IgG1 [34]. It also counterbalances the Th1 phenotype (mediated by IFNγ and TNFα) and subsequent activation of cell-mediated immunity and phagocytic activity [36].

Table 3. Multivariate analysis of factors associated with *Pneumocystis jirovecii* pneumonia.

	Discovery study ^a , N = 1424			Replication study ^a , N = 1825			All patients ^a , N = 3210		
	SHR	95% CI	P ^a	SHR	95% CI	P ^a	SHR	95% CI	P ^a
Age ^b	1.00	0.99–1.02	0.8	1.01	1.00–1.02	0.06	1.01	1.00–1.02	0.1
Male sex	0.78	0.53–1.15	0.2	0.95	0.69–1.30	0.7	0.87	0.68–1.11	0.3
CD4 ⁺ slope ^c	0.17	0.11–0.26	<0.0001	0.16	0.11–0.24	<0.0001	0.17	0.13–0.22	<0.0001
Maximal HIV RNA (log copies/ml)	1.37	1.11–1.69	0.003	1.67	1.38–2.01	<0.0001	1.53	1.33–1.76	<0.0001
Type of HIV transmission									
MSM	Ref.			Ref.			Ref.		
Heterosexual	1.22	0.87–1.71	0.2	0.98	0.72–1.34	0.9	1.09	0.87–1.37	0.5
Intravenous drug use	1.00	0.61–1.63	1.0	0.72	0.49–1.04	0.08	0.85	0.63–1.14	0.3
Other	1.64	0.92–2.91	0.09	1.27	0.77–2.11	0.3	1.39	0.96–2.03	0.09
Cohort entry (years)									
<1995	Ref.			Ref.			Ref.		
1995–2000	0.98	0.70–1.38	0.9	0.83	0.60–1.14	0.3	0.90	0.71–1.13	0.4
2001–2005	0.77	0.49–1.20	0.2	0.92	0.66–1.27	0.6	0.90	0.70–1.15	0.4
>2005	0.32	0.04–2.63	0.3	0.20	0.06–0.69	0.01	0.25	0.09–0.69	0.008
PJP prophylaxis ^d	0.44	0.32–0.60	<0.0001	0.31	0.24–0.40	<0.0001	0.36	0.30–0.44	<0.0001
ART/HAART ^e	0.79	0.75–0.83	<0.0001	0.75	0.71–0.80	<0.0001	0.77	0.74–0.80	<0.0001
HCV coinfection ^e	0.93	0.87–1.00	0.05	0.94	0.89–0.99	0.01	0.93	0.90–0.97	0.001
Tobacco smoking ^f	0.77	0.57–1.03	0.08	0.89	0.68–1.15	0.4	0.83	0.68–1.01	0.06
IL-4 rs2243250 TT/TC versus CC ^g	1.43	1.07–1.92	0.02	1.42	1.08–1.85	0.01	1.42	1.17–1.73	0.0004

ART, antiretroviral treatment; CI, confidence interval; HCV, hepatitis C virus; OR, odds ratio; PJP, *Pneumocystis jirovecii* pneumonia; SHR, subhazard ratio (competing risk regression).

^aVariables potentially associated with *Pneumocystis carinii* pneumonia (cut-off $P < 0.1$ by univariate testing, Supplemental Table 2, <http://links.lww.com/QAD/B490>) were entered into the multivariate analysis, with age and sex forced into the model. The number of patients is slightly lower than the number of patients included in the studies because some covariables are missing for some patients (refer to Table 1 for details).

^bAt estimated HIV infection date (refer to Methods section); SHR is calculated per 1 additional year of age.

^cRate of CD4⁺ depletion before HAART (refer to Methods section); Note: similar results were found when CD4⁺ were accounted for as a time-dependent covariates (refer to Supplemental Table 3, <http://links.lww.com/QAD/B490>).

^dAt any time during follow-up.

^eTime-dependent covariates.

^fAt cohort entry: more than 10 U packet-year.

^gGenetic associations are for the dominant mode of inheritance (patients homozygous and heterozygous for the rare allele are compared with the other). Because some genotypes were missing for rs2243250 in the discovery study, the association was also run for rs2070874, which is in strong LD with rs2243250 ($R^2 = 0.96$): OR = 1.37, 95% CI 1.05–1.80, $P = 0.02$ (model including 1629 patients).

A number of studies have shown that immunity against *Pneumocystis* spp. is mediated by both Th1 and Th2 responses [37]. Inhibition of the Th1 response by using anti-TNF α antibodies induced decreased [36] or delayed [38] pathogen clearance in two different mice models of PCP. Reversely, stimulation of Th1 responses by using an adenoviral vector encoding IFN γ protected T cells depleted mice from PCP [39] and recombinant IFN γ increased survival in a rat model of PCP. Inhibition of B cells in mice by using antibodies targeting CD20 also leads to increased susceptibility for PJP [40]. The risk of *Pneumocystis* spp. infections in humans is increased in patients with primary immune deficiencies, such as X-linked hyper-IgM syndrome [41], as well as in patients treated with monoclonal antibodies against the CD20+ antigen on B cells (rituximab or obinutuzumab [42,43]), the CD52 antigen on B and T cells (alemtuzumab [44]), or with Bruton's tyrosine kinase inhibitor (ibrutinib [45]).

Several studies suggested that the presence of the -590T allele in rs2243250 is associated with increased serum or plasma IL-4 levels [46–49], although this was not universally confirmed [50,51]. Higher IL-4 gene expression may result

from a new binding site for nuclear factor of activated T cells, the main transcription factor for the IL-4 expression, at the nucleotide position -590 (Supplemental Fig. S2, <http://links.lww.com/QAD/B490>) [52]. Conversely, the -590T allele was associated with reduced IFN γ and TNF α expression and/or production by human immune cells stimulated with phorbol myristate acetate/Ionomycin (including neutrophils, monocytes and lymphocytes), suggesting that higher IL-4 production could counteract Th1 responses, leading to decreased *Pneumocystis* spp. clearance [46].

Altogether, this data suggest that increased IL-4 levels in -590T allele carriers result in increased susceptibility to infections mainly as a results from reduced Th1 responses, and that this defect cannot be adequately compensated by a concomitant or subsequent increase in Th2 responses. Consistent with this hypothesis, the -590T allele was associated with an increased risk of vulvo-vaginal candidiasis, as well as increased vaginal IL-4 levels, in a cohort of 85 Latvian women [53] and a higher risk of paracoccidioidomycosis in a cohort of 81 Brazilian individuals [51]. In a cohort of adult leukemia patients,

the -590T allele was protective for hepatosplenic candidiasis, as a possible result of diminished immune reconstitution after neutropenia [20]. In addition, numerous studies associated the -590T variant with susceptibility to pathogens other than fungi, such as respiratory syncytial virus (RSV) [54–57], *Plasmodium falciparum* [58], *Brucella* spp. [59], *Clostridium difficile* [60] and bacteria causing periodontitis [61–65].

Also consistent with this hypothesis, animal studies showed that IL-4 deficiency is associated with protection against fungal, mycobacterial and parasitic infections. In a cyclophosphamide-induced mice model of invasive aspergillosis, mice deficient in IL-4 had increased survival [66] and increased broncho-alveolar lavage IFN γ levels, compared to WT mice. In a mouse model of tuberculosis, IL-4-deficient mice had decreased disease severity and increased TNF α lung expression compared to WT mice [67]. In a mouse model of RSV infection, overexpression of IL-4 was associated with decreased viral clearance and neutralization of IL-4 with a reduced illness score [68,69]. In a murine model of *Leishmania major* infection, parasite clearance was positively correlated with the production of IFN γ (Th1) and negatively correlated with that of IL-4, IL-5 and IL-13 (Th2) [70].

Like other genetic association studies, our study has some limitations. The date of HIV-1 infection was estimated by using a joint back calculation model in seroprevalent patients [31]. Although it is by far the largest association study for PJP infection, our study may have failed to detect associations with rare variants, such as those in Dectin-1 (MAF=0.08), Toll-like receptor 1 (TLR1) (MAF=0.08) or TLR4 (MAF=0.05), which have been associated with susceptibility to infections due to other fungi. Our study did not replicate a previously reported association with MBL2 low expression haplotypes [21], despite reasonable power to do so (>80% power to detect an association with hazard ratio=1.5; Supplemental Table S1, <http://links.lww.com/QAD/B490>). Despite substantial evidence for a role for rs2243250 on IL-4 production, baseline IL-4 levels have not been measured in study patients to further support genetic associations. In addition, while the SHCS is a well established longitudinal cohort with robust follow-up, patients management strategies including prophylaxis and antiretroviral treatment have been evolving over year. Yet, despite the limitations, association with IL-4 SNP was still significant in multivariate models accounting for prophylaxis and different periods of cohort entry.

In conclusion, this data demonstrates an association between PJP and the presence of the interleukin-4-590T/C polymorphism in a large cohort of HIV patients. This SNP may influence the Th2/Th1 responses required for appropriate immunity against *Pneumocystis* spp. and increase susceptibility to infection in HIV-positive patients with low level of CD4⁺ T cells.

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Conflicts of interest

There are no conflicts of interest.

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